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GLUCOSE METABOLISM IN LIVER AND AN ADENOCARCINOMA OF MICE WITH AND WITHOUT HYPERTHERMIA

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Abstract

Glycolytic metabolites such as glucose, glucose-6-phosphate, fructose-1.6-diphosphate, dihydroxyacetone-phosphate, glycerol-3-phosphate, pyruvate and lactate as well as acetoacetate and ß-hydroxybutyrate were measured in mouse liver and adenocarcinoma after local hyperthermia for one hour at 43 ^{o}C and a glucose load of 6 mg/g body weight.

The combined treatment led to an increase of the lactate level, at the same time the glucose degradation was apparently enhanced. The redoxequilibria were shifted to the reduced metabolites. This could be interpreted that hypoxia was induced or enhanced, which could have significance for the tumor therapy.

At later periods after hyperthermia a metabolic situation occurred which was also observed in severe diabetes. Such alterations which occurred also in the liver must be considered in connection with normal tissue damage.

Introduction

Metabolic pathways are very well regulated. Temperature changes can alter the rate of enrymatic reactions which can lead to disorders of the whole pathway. At very high temperatures denaturation of proteins occurs (1). Damage to cancer cell metabolism by hyperthermia has been reported (2,3). Von Ardenne has suggested that the heat sensitivity of cancer cells can be increased by glucose (4,5). It is assumed that lactate accumulates under these conditions and induces a decrease of the intracellular pH-value.
Other authors have shown that the lowering of the pH-value causes a remarkable enhancement of cell killing by hyperthermia (6).
Glucose metabolism is expremely complex and coupled in several ways with the described parameters. Therefore it seemed of interest to study glucose metabolism and its alteration by hyperthermia in an experimental tumor. Tumor therapy is very strongly linked to the tolerance of normal tissues. As glucose metabolism is especially active in liver, hepatic tissue is included in these studies.

Materials and Methods

Inbred mice (C_{57} black) were used for all experiments. An adenocarcinoma was transplanted into these animals by injection of a tumor cell suspension into the muscles of the hind leg. Six days after the transplantation the animals were used for the experiments. For the studies of hyperthermia the hind leg with the tumor was heated in a water bath for one hour at 43 ^{o}C. During heating the animals were kept unter anaesthesia. In some animals the temperature was measured in the tumor and in the neck of the animals. Within 10 minutes the temperature increased from 35.2 ^{o}C to 42.0 ^{o}C in the tumor, after 30 minutes heating it reached a value of 42.7 ^{o}C and stayed at this temperature until the end of heating. After the heating the temperature decreased in the tumor to less than 30 ^{o}C

and remained at this low level for several hours. In the neck the temperature also increased to 40.4 °C within 30 minutes of heating at 43 °C. After the hyperthermic treatment the rectal temperature decreased to about 32 °C for several hours.

For the metabolic determinations the liver tissue and tumor were taken off under anaesthesia, homogenized with 4 percent perchloric acid and centrifuged. The supernatant was neutralized and used for the determination of glucose, glucose-6-phosphate (G-6-P), fructose-1.6-diphosphate (FDP), dihydroxy-acetonephosphate (DAP), glycerol-3-phosphate (G-3-P), pyruvate, lactate, acetoacetate (AcAc) and ß-hydroxybutyrate (ß-HO-Bu). The metabolites were measured by enzymatic assays coupled to NAD^+ or NADH.

Glucose was injected intraperitoneally directly before the hyperthermia treatment (6 mg/g body weight).

Results and Discussion

After the intraperitoneal injection of glucose in a rather high dose (6 mg/g body weight ≙ 33.4 µ moles/g) the glucose level increased in liver tissue very rapidly and within 10 minutes reached its highest level. 20 minutes after injection the glucose level was about the same, 60 minutes after administration it had decreased again (Table 1). Most of the measured glycolytic metabolites were not considerably altered in the hepatic tissue. Only pyruvate and lactate, the end products of glycolysis were enhanced.

When the local heating of the hind leg with the tumor (one hour at 43 °C) was started just after the glucose injection the glucose content was the same in the liver as without hyperthermia. However the levels of glycerol-3-phosphate, pyruvate, lactate and also ß-hydroxybutyrate had increased under these conditions. As mentioned earlier the local heating induced a rise of the temperature in the whole mouse to about 40 °C (Table 1). These data demonstrate the difficulties in establishing a local heating of tissues and as a

consequence in localizing the effects of hyperthermia to the tumor. The problem of heat dissipation is especially acute with smaller animals such as the mouse and will be less serious in clinical tumor therapy, although it also exists.

In the tumor tissue the glucose content increased at about the same rate as in the liver. The highest levels were obtained 20 - 40 minutes after the injection (about 14 µ moles/g tissue). The incrase of the lactate content was comparatively small in the tumor. A similar effect was observed for the levels of FDP and DAP (Table 2).

Hyperthermia led to a decrease of the glucose content in the tumor, also G-6-P was reduced. On the other hand an increase of G-3-P and ß-hydroxybutyrate was observed directly after hyperthermia. Similar changes occurred when a glucose load was given to the animals just before the hyperthermia treatment was started. However under these conditions (glucose plus hyperthermia) the lactate content was also enhanced in the tumor directly after the hyperthermia treatment had ended.

Dickson and Calderwood (8) observed an inhibition of glycolysis in Yoshida sarcoma of rats after hyperthermia treatment in vivo when the tumor tissue was incubated in vitro. Our data appeared not to support such a mechanism for the period during hyperthermia. The finding that the glucose level was lowered by hyperthermia demonstrated a more rapid glucose turnover. In other experiments it could be shown that the output of $^{14}CO_2$ by mice was increased during whole body hyperthermia (1 hour at 40 ^{o}C or 41 ^{o}C) after injection of radioactively labelled glucose (unpublished results). Further it has been shown that during whole body hyperthermia a considerable breakdown of hepatic glycogen occurred and no accumulation of glycolytic metabolites was found (9). However some hours after hyperthermia the metabolic degradation of glucose was apparently decreased. This effect was in agreement with the data of Dickson and Calderwood(8) and will be discussed later. It was further surprising that most of the glycolytic metabolites increased only to a small extent besides

lactate. A higher rise of G-6-P, FDP and DAP was seen in rats with a transplantable glioblastoma after chronic glucose infusion which coincided with a decrease of the pH-value (unpublished results). These data suggested that if an inhibition of glucose degradation occurred by hyperthermia it may be that the lactate consumption was somewhat reduced.

Also it should be mentioned that hyperthermia plus glucose shifted the redoxequilibria into the direction of the reduced metabolites (Table 1 and 2). This occurred for both the ratios of lactate/pyruvate and ß-hydroxybutyrate/acetoacetate especially in the tumor. It could mean that hypoxia was induced or enhanced under these conditions. It further could have some relevance to the pH-value through the following equation:

$$K = \frac{[\text{lactate}] \times [NAD^+]}{[\text{pyruvate}] \times [\text{NADH}] \quad [H^+]}$$

Analogous equations are valid for the other redoxequilibria. Such changes would have a great significance for tumor therapy and must be investigated in the future.

In further experiments the metabolites were measured over a period of several hours after hyperthermia treatment without glucose injection (Table 3). A strong decrease of the glucose content was observed which was followed by the other glycolytic metabolites including lactate and pyruvate. During this period the CO_2-output from glucose was also diminished (unpublished results). However, two other strong acidic metabolites, acetoacetate and ß-hydroxybutyrate were greatly increased after hyperthermia. Similar observations were made in the liver tissue of mice after whole body hyperthermia(9). Thus a metabolic situation occurred which was also found in severe diabetes. The acidic metabolites could be formed from glucose via lactate and acetyl-CoA. These metabolic alterations might also contribute to the liver damage which was observed after clinical application of hyperthermia.

Thus glucose matabolism might be interesting for two reasons: On the one hand the sensitivity of the tumor tissue to hyperthermia might be altered. Blood flow and concomittantly the oxygen supply can be influenced. On the other hand hyperthermia could induce changes of the glucose metabolism for a longer period and these effects could contribute to pathophysiological damage in normal tissues.

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Table 1

	Control	Glucose 10 minutes	Glucose 60 minutes	Glucose and Hyperthermia 60 minutes
Glucose	10.2 ± 0.2	37.0 ± 3.0	23.9 ± 2.0	23.4 ± 0.1
G-6-P	0.36 ± 0.01	0.42 ± 0.02	0.36 ± 0.02	0.32 ± 0.02
FDP	0.038 ± 0.003	0.031 ± 0.002	0.041 ± 0.02	0.034 ± 0.002
DAP	0.035 ± 0.001	0.035 ± 0.002	0.043 ± 0.02	0.038 ± 0.002
G-3-P	0.52 ± 0.02	0.51 ± 0.02	0.70 ± 0.05	1.35 ± 0.06
Pyruvate	0.047 ± 0.003	0.119 ± 0.011	0.10 ± 0.01	0.13 ± 0.01
Lactate	1.95 ± 0.09	3.29 ± 0.15	3.67 ± 0.13	6.10 ± 0.17
AcAc	0.049 ± 0.004	0.053 ± 0.007	0.061 ± 0.005	0.060 ± 0.005
ß-HO-Bu	0.19 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.32 ± 0.02
G-3-P/DAP	14.8	14.6	16.3	35.5
Lact/Pyruvate	41.5	27.5	36.7	47.0
ß-HO-Bu/AcAc	3.9	3.0	2.8	5.3

Content of metabolites (µ moles/g tissue) in mouse liver after i.p. injection of 6 mg/g body weight glucose and hyperthermia for one hour.

Table 2

	Control	Hyperthermia directly after Hyperthermia	Glucose 60 minutes	Glucose + Hyperthermia 60 min. after injection directly after hyperthermia
Glucose	3.12 ± 0.10	1.19 ± 0.14	10.96 ± 1.19	7.99 ± 0.47
G-6-P	1.02 ± 0.03	0.65 ± 0.04	0.70 ± 0.03	1.10 ± 0.10
FDP	0.030 ± 0.001	0.027 ± 0.003	0.048 ± 0.002	0.061 ± 0.002
DAP	0.036 ± 0.001	0.030 ± 0.002	0.046 ± 0.002	0.054 ± 0.002
G-3-P	0.37 ± 0.01	0.50 ± 0.02	0.36 ± 0.01	0.58 ± 0.01
Pyruvate	0.22 ± 0.01	0.22 ± 0.01	0.20 ± 0.01	0.17 ± 0.01
Lactate	14.3 ± 0.3	13.1 ± 0.5	16.5 ± 0.5	26.5 ± 0.6
AcAc	0.017 ± 0.002	0.016 ± 0.003	0.018 ± 0.002	0.014 ± 0.002
ß-HO-Bu	0.097 ± 0.007	0.159 ± 0.008	0.069 ± 0.004	0.15 ± 0.01
G-3-P/DAP	10.2	16.7	7.8	10.4
Lact/Pyruvate	65	60	82	156
ß-HO-Bu/AcAc	5.7	10.0	3.8	10.7

Content of metabolites (µ moles/g tissue) in transplanted adenocarcinomas after i.p. injection of 6 mg/g body weight glucose and hyperthermia for one hour.

Table 3

	Control	1 hour	6 hours	12 hours
Glucose	3.12 ± 0.10	1.59 ± 0.12	1.35 ± 0.08	1.04 ± 0.064
G-6-P	1.02 ± 0.03	0.70 ± 0.04	0.90 ± 0.046	0.44 ± 0.036
FDP	0.030 ± 0.001	0.022 ± 0.002	0.027 ± 0.001	0.017 ± 0.001
DAP	0.036 ± 0.001	0.029 ± 0.002	0.029 ± 0.002	0.023 ± 0.002
G-3-P	0.37 ± 0.01	0.31 ± 0.01	0.37 ± 0.02	0.28 ± 0.012
Pyruvate	0.22 ± 0.01	0.24 ± 0.01	0.17 ± 0.01	0.11 ± 0.01
Lactate	14.3 ± 0.3	11.9 ± 0.4	12.1 ± 0.4	7.90 ± 0.30
AcAc	0.017 ± 0.002	0.041 ± 0.007	0.058 ± 0.009	0.19 ± 0.02
ß-HO-Bu	0.097 ± 0.007	0.249 ± 0.027	0.338 ± 0.030	0.75 ± 0.07
G-3-P/DAP	14.8	10.7	12.8	12.1
Lact/Pyr	41.5	49.6	36.7	46.7
ß-HO-Bu/AcAc	3.9	6.1	5.9	4.9

Content of metabolites (µ moles/g tissue) in transplanted adenocarcinomas after hyperthermia for one hour.